Remarks/Arguments

Claims 1-18 and 20-22 are pending in the application. Claims 1-13 and 20-22 have been withdrawn from consideration pursuant to a lack of unity objection. Claims 14-18 are under consideration.

Election / Restriction Requirement

Reconsideration and withdrawal of the lack of unity objection as to all claim groups is respectfully requested. As pointed out in the Response to Restriction Requirement, the special technical feature of the invention is an immunogenic determinant, which comprises a prokaryotic cell which has been treated in order to increase the amount of trehalose present within the cell.

The Examiner considers the special technical feature which links groups I-IV of the claims as being a method of modifying cells to increase the production of trehalose. The Examiner further submits that the cited prior art teaches of such a method.

The cited prior art does teach of cells in which trehalose production has been induced. However, the cited prior art does not disclose or suggest that these cells may be more immunogenic than non-induced cells.

Further, the aim of the prior art is to provide a cell which is both *dried* and *stabilized*. The cells of the present invention as claimed do not need to be dried. They also do not need to be stabilized.

As acknowledged at page 2, lines 1 through 5 and page 2, lines 26 through 29 of the present application, prokaryotic cells are stabilized through the drying of the cells in a carbohydrate matrix following the induction of trehalose synthesis. Accordingly, trehalose production is induced *inside* the cell, while a carbohydrate glass former (such as trehalose) is used outside of

the cell when drying. Example 5 in particular teaches of the use of prokaryotic cells which have had trehalose induced within them, but which are not dried. Drying is not a step required in order to produce the immunogenic determinant of the present invention, further trehalose is not required in the drying medium, where a drying medium is used.

It is therefore submitted that the immunogenic determinants as claimed in the instant application is structurally different to the dried, stabilized prokaryotic cells of the prior art.

Specification

In response to section 7 and 8 of the Detailed Action, the specification has been amended to change the spelling of "immunised" and "immunisation" to *immunized* and *immunization*, respectively. The term 'Quil A' has been capitalized with "TM" placed in superscript thereafter to denote its use as a Trade Mark. The term 'Detox' has been capitalized with "TM" placed in superscript thereafter to denote its use as a Trade Mark

Priority

Examiner's request for amendment of the specification to include a reference to an earlier application is incorrect. The prior applications which must be referenced are prior US nonprovisional applications from which internal priority is claimed pursuant to 35 USC 120 (continuations, continuations-in-part, and divisionals), or US provisional applications, from which priority is claimed pursuant to 35 USC 119(e). The instant application comprises the US national phase of a PCT application. It is not a continuation, continuation-in-part, or divisional of an earlier US application; nor does it claim the benefit of the filing date of an earlier US provisional application. Thus, amendment of the specification is not required under Rule 78.

Response to Section 112, 1st paragraph Rejection

Examiner alleges that while the specification, while being enabling for induction of trehalose synthesis in some strains of bacteria and production of antibodies in animals against said strains, does not reasonably provide enablement for a vaccine. Examiner submits that the specification does not enable any person skilled in the art to which it pertains, or with which it is connected to, to make and/or use the invention commensurate with the scope of the claims.

Applicant submits that the provision of a vaccine is provided by the teaching of the present invention.

With regard to the discussion of enablement, it is pointed out that assessment of enablement is not based on whether any experimentation is necessary to perform the scope of the invention as defined in the claims, but rather whether the level of that experimentation is undue. Importantly, the fact that experimentation may be complex does not make it undue.

In the present case, the person of ordinary skill in the art would be substantially qualified and experienced in the field, having at least a post-doctoral level of qualification.

Techniques for inducing trehalose production in prokaryotic cells are discussed in the description at page 5, line 26 through page 6, line 16. This section is preceded by a discussion of the mechanisms of trehalose production in prokaryotic cells and in particular, bacteria and yeast.

At page 6, line 32, the description teaches of a preferred method for inducing trehalose production in the bacteria *Escherichia*. In particular, specific conditions for osmotic shock, which will in turn result in trehalose production are described.

Although Example 1 is directed to trehalose production in *E.coli* using osmotic shock, page 5 line through page 10, line 4 discuss generally a number of ways in which trehalose can be

induced, this discussion including a consideration of the specific cell type. This provides a clear demonstration that the description acknowledges the diversity of cellular forms encapsulated by the term "prokaryotic cells" (a term defined at page 5, line 3 of the description).

In particular, the paragraph at page 9, line 27 reads "The time required for stimulating trehalose synthesis depends, inter alia, on the nature of the procaryotic cells (including genus, species, and/or strain) and the conditions under which trehalose induction occurs (i. e., whether by osmotic shock, oxygen deprivation, etc.). For trehalose induction by osmotic shock, the time required for maximum concentration of trehalose in turn depends on the degree of osmolarity as well as the particular salts used. The optimum conditions for trehalose synthesis can readily be determined by simple trial and errors tests". This passage teaches the skilled man that the conditions required for trehalose induction are dependent upon the cell type in which trehalose is being induced.

A vaccine composition in accordance with the present invention as described at page 4, line 8 is defined as containing the immunogenic determinant of the invention, this being a treated prokaryotic cell in which trehalose production has been induced. Line 19 of page 4 further teaches that the vaccine composition will contain an adjuvant and be provided in an aqueous carrier medium and be administered by injection.

Suitable adjuvants are listed at page 10, line 29. Specific discussion relating to the route of administration of the vaccine is provided at page 11, line 31. These routes of administration are standard in the art and would be known to the skilled man.

The concentration of the immunogenic determinant (prokaryotic cell in which trehalose production has been induced) is discussed at page 12, line 8. Specific dose range levels in relation to body weight of the human or animal to be treated with the resulting vaccine are provided.

Dosage regimen is discussed in the description at page 12, line 11 through line 17. Vaccination is used in order to induce an immune response which confers long term protective immunity. The above-cited passage of the description provides teaching in order to facilitate the establishment of this immunological memory. Specifically, the description teaches that the following an initial inoculation of a human or animal with the vaccine, subsequent repeat 'booster' inoculations can be provided. These booster doses can be at the same or different doses and are administered over a period of 1 to 26 weeks from the date of initial inoculation. The effect of administering such repeat doses will be to induce immunological memory which will allow long term protective immunity to result.

The Examples provided in the description specifically exemplify and further describe the vaccination procedure.

In Example 1, trehalose production is induced in *E.coli* through the provision of osmotic shock. Although osmotic shock is exemplified, the prior teachings provided in the description and referred to above would allow other mechanisms of stress to be applied in order to result in trehalose production.

In Example 2, trehalose production was induced in *Salmonella typhimurium* using osmotic shock. The level of salt concentration was the same as that used in Example 1. Of course, page 7, lines 28-30 has taught the skilled reader that the level of salt concentration (osmolarity) required to induce trehalose synthesis will be dependent upon the genus, species and / or strain of the prokaryotic cell, hence the salt levels described in Examples 1 and 2 may vary.

Example 3 teaches of the cells of Examples 1 and 2 having trehalose synthesis induced therein. The induced cells are then stabilized using a drying solution (which contains amongst other

things, trehalose). The use of the drying solution is not essential to the use of the induced cells in a vaccine preparation.

Example 4 describes the use of the cells prepared in Example 3 as vaccines. Vaccination of mice and rabbits with dried and un-dried preparations of the cells of Examples 1 and 2 was performed and the magnitude of the immune response assessed. In each of the cases (dried and undried) an immune response was shown to result and accordingly, vaccination was effective at conferring protective immunity.

Example 5 exemplifies the use of heat in order to induce trehalose production. *E.coli*, *Salmonella typhimurium* and *Mycobacterium bovis* were heat shocked. This heat shocking resulted in a 3-5 fold increase in trehalose within the cells.

The induced bacteria were killed such that a killed vaccine (rather than a live vaccine resulted). Repeated freeze-thaw cycles were used to kill the cells, however the cells may be killed in other ways as discussed at page 10, line 15.

The induction of immunity following immunization was measured by performing an assay of the antibody titres. The production of antibodies is key to inducing an immune response which has 'memory' such that lasting protection is conferred against the pathogen which was used to derive the antigenic component in the vaccine (against which the immune response is directed).

It should be noted that the cells which are used to vaccinate the rabbits were not dried. As such, in order to induce the improved immune response, only trehalose induction was required and not trehalose induction followed by drying in a medium containing trehalose.

The conserved nature and function of trehalose across the spectrum of prokaryotes means that the induction of trehalose can be expected to result in the same enhancement in immunogenicity as seen in the bacteria which are described in the Examples.

Accordingly, the principle which is taught by the present invention of increasing the immunogenicity of a prokaryotic cell by inducing trehalose production within it, could be expected to be achieved in all prokaryotes. Although the specific mechanisms and stimuli which are required to induce trehalose production can differ between individual prokaryotes, the description acknowledges this and provides the reader with a number of approaches through which trehalose induction can be achieved. The trehalose which is induced however serves to have a consistent effect on the prokaryotic cells of increasing their immunogenicity.

It is therefore submitted that the Applicant's invention is enabled for the prevention, amelioration, or treatment of all bacterial and pathogenic diseases as claimed due to the conserved function of trehalose and the consistent effect of induced immunogenicity which results in prokaryotic cells in which trehalose has been induced.

Turning to the Examiner's comments at the foot of page 6 with regard to the correlation of the vaccine of the present invention with equivalent results in humans, Applicant respectfully submit that the 1989 scientific journal paper cited by the Examiner in support of the rejection does not actually reflect a state of art at the priority filing date of the present application namely, August 1999.

Submitted herewith a copy of the 1998 paper, Baldwin et al., "Evaluation of New Vaccines in the Mouse and Guinea Pig Model of Tuberculosis", *Infection and Immunity, 66(6)*:2951-2959, which exemplifies vaccine models in the mouse and guinea pig in relation to immunity from tuberculosis.

Baldwin et al. demonstrates that vaccination in the animal model can be used as a basis from which to extrapolate equivalent expected results in the human model. The paper serves to confirm that the skilled artisan in the field would understand this as being so.

It is further pointed out that an application to the NIAD which included only data for a tuberculosis vaccine conducted on animals would be considered by the NIH to provide sufficient information in order to consider the efficacy of that vaccine. The Journal paper of Brennan et al., "Propelling novel vaccines directed against tuberculosis through the regulatory process", *Tubercle and Lung Disease (1999) 79(3)* 145-151 provides specific guidance on this. The author, Michael Brennan is of the FDA (Food and Drug Administration). The article provides guidance on the procedure for the development and testing of new tuberculosis vaccines and specifically how new tuberculosis vaccines can be progressed through clinical trials.

Details relating to the NIH's policy relating to the use of animal models for vaccine testing for tuberculosis can be found at URL: http://www.niaid.nih.gov/dmid/tuberculosis/#opp.

The section headed "Tuberculosis Drug Development Resources" links to information on NIH sponsored facilities for vaccine testing in animal models. The NIAID sponsored site can be directly accessed at URL: http://www.taacf.org/.

The direct URL for one such facility is:

http://www.cvmbs.colostate.edu/microbiology/tb/mission.html

and also:

http://www.cvmbs.colostate.edu/microbiology/tb/VT1.html.

Response to Section 112, 2nd paragraph Rejection

The rejection of claim 15 is overcome by explicitly incorporating the method of claim 1 into claim 15.

The rejection of claim 18 is overcome through the amendment made herein.

Response to Section 102 Rejection

Claims 14-18 have been rejected as being anticipated by Tunnicliffe et al. (WO 98/24882)

WO 98/24882 relates to methods of drying and stabilizing cells by inducing intracellular trehalose production. The induction of trehalose facilitates the storage of the induced cells. The drying and stabilizing process further facilitates the subsequent rehydration of the cells.

As acknowledged at page 2, lines 1 through 5 and page 2, lines 26 through 29 of the present application, prokaryotic cells are stabilized through the drying of the cells in a carbohydrate matrix following the induction of trehalose synthesis. Accordingly, trehalose production is induced *inside* the cell, while a carbohydrate glass former (such as trehalose) is used outside of the cell when drying. Accordingly, WO 98/24882 teaches that in order to fully exploit the stabilizing properties of trehalose that it is necessary to endure that trehalose is present both inside and outside of the bacterial cells. The presence of trehalose both within and without the cell was considered to be necessary in order to prevent desiccation of intracellular or extracellular structures of the cell.

The aim of the prior art is to provide a cell which is both *dried* and *stabilized*. Drying is facilitated by mixing the cells with a drying agent which includes a stabilizing agent such as trehalose. The cells of the present invention as claimed do not need to be dried in the presence of trehalose.

The cited prior art does teach cells in which trehalose production has been induced. However, the cited prior art does not disclose or suggest that these cells may be more immunogenic than non-induced cells.

The discussion in the prior art of the use of the stabilized cells in relation to their use in vaccines does not relate to the fact that the stabilized cells are more immunogenic. Rather, the authors

have tried to predict uses for the stabilized cells which they have produced. One such identified use is to utilize the improvement in storage provided by the invention to allow live bacterial vaccines to be produced. In such a case, the bacteria cells will have trehalose induced, but will also be dried in the drying medium. This allows the cells to be live. The present invention is not restricted to using live cells in the vaccine preparations claimed, both killed and live vaccines may be provided (page 10, line 15).

As acknowledged at page 2, lines 1 through 5 and page 2, lines 26 through 29 of the present application, prokaryotic cells are stabilized through the drying of the cells in a carbohydrate matrix following the induction of trehalose synthesis. Accordingly, trehalose production is induced *inside* the cell, while a carbohydrate glass former (such as trehalose) is used outside of the cell when drying. Drying is an essential step (see page 6, line 21 of WO 98/24882).

Example 10 of WO 98/24882 relates to the stabilization of *Salmonella* with trehalose. The stabilized cells are dried (see p33, line 23).

The step of drying the cells using a drying medium in which trehalose is present is not required in the present invention. Page 3, line 7 of the description of the present invention reads "we have also found that this increased immunogenicity of the stabilised procaryotic cells is not dependent on the drying process considered essential in the above stabilisation processes, but results from the induction of trehalose synthesis". Further, Example 5 of the present invention in particular teaches of the use of prokaryotic cells which have had trehalose induced within them, but which are not dried. Drying is not a step required in order to produce the immunogenic determinant of the present invention. Where the cells are dried, trehalose is not present in the drying medium.

The exclusion of trehalose from the drying medium is not taught or considered in the prior art. More specifically, the presence of trehalose both inside and outside of the cell at the time of drying is considered to be essential.

It is therefore submitted that the immunogenic determinants as claimed in the instant application is structurally different to the dried, stabilized prokaryotic cells of the prior art.

Claims 14-18 are further rejected as being anticipated by Tunnicliffe et al.(US 6,468,782).

This US Patent claims a common priority to that of WO 98/24882 which is considered above. The content of these documents is substantially the same.

Accordingly the comments made in relation to the WO 98/24882 provided above are referred to. It is submitted that a definite structural difference exists between the stabilized prokaryotic cells of US 6,468,782 and those of the present invention.

Reconsideration and withdrawal of the Section 102 rejection is respectfully submitted.

Conclusion

The claims of the application are believed in condition for allowance. An early action toward that end is earnest solicited.

Respectfully submitted

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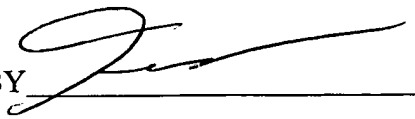
BY 

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